

REMARKS

Applicants respectfully request the Examiner to reconsider the merits of the objections and rejections in view of the foregoing amendment and the following remarks.

Upon entry of the foregoing amendment, claims 291-295 are pending in the application.

Claims 287 and 290 are canceled.

Claims 296-303 have been allowed.

Applicants respectfully request entry of the above amendment and submit that the above amendment does not constitute new matter.

Claim Rejections under 35 USC §102

Claims 287, 290, 292 and 293 stand rejected under 35 U.S.C. 102(b) as being anticipated by Smith et al. (JACS, a983, Vol. 105, pp 6638). The Examiner stated the following:

Smith et al. discloses a nonmetallic porphyrin comprising an aldehyde moiety as a reactive group attached to the non-pyrrole position. (see page 6639, structure 36). Said porphyrin comprises alkyl group attached to the pyrrole ring, which can compromise further a polar group. It is well known in the art that an aldehyde is capable of forming a carbon-carbon linkage.

Compound 36 of Sinclair et al. shows an aldehyde in a non-pyrrole position of a non-metallic porphyrin. Applicants have deleted aldehyde as a potential R_o group in Claim 291. The newly amended claim is therefore not anticipated by Smith et al.

Jannis Stavrianopoulos et al.

Serial No.: 10/763,088

Filed: January 22, 2004

Page 10 Reply and Amendment in Response To July 11, 2008 Office Action – January 9, 2009

Claims 291, 294 and 295 were objected to as being dependent upon a rejected base claim.

Applicants have amended the claims to overcome these rejections. Specifically, the claims have been rewritten in independent form and amended to include the limitations of the base claims and any intervening claims. No new matter has been added by these claim amendments. Applicants believe that the amendments made to the claims now place the claims in condition for allowance.

Jannis Stavrianopoulos et al.

Serial No.: 10/763,088

Filed: January 22, 2004

Page 11 Reply and Amendment in Response To July 11, 2008 Office Action – January 9, 2009

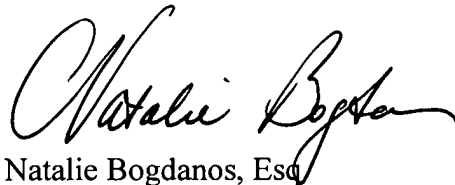
SUMMARY

Applicants respectfully request reconsideration and withdrawal of the rejections and objections of record and further examination of the amended claims. These claim amendments have not resulted in the addition of new matter. Early and favorable action is respectfully requested.

No other fee or fees are believed due in connection with this paper. In the event that any fee or fees are due, however, the United States Patent and Trademark Office is hereby authorized to charge any such fee or fees to Deposit Account No. 05-1135, or to credit any overpayment thereto.

If a telephone conversation would further the prosecution of the present application, Applicants' undersigned attorney requests that she be contacted at the number provided below.

Respectfully submitted,



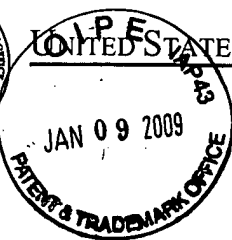
Natalie Bogdanos, Esq.
Registration No. 51,480
Attorney for Applicants

ENZO LIFE SCIENCES, INC.
c/o ENZO BIOCHEM, INC.
527 Madison Avenue, 9th Floor
New York, New York 10022-4304
Telephone: (212) 583-0100
Facsimile: (212) 583-0150

al/USProsecution/Enz-61(D5).replytoOA.1.9.09



UNITED STATES PATENT AND TRADEMARK OFFICE

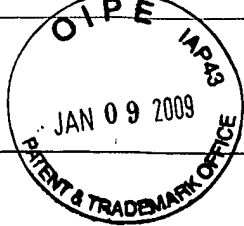


UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/763,088	01/22/2004	Jannis G. Stavrianopoulos	Enz-61(D5)	6065
<div>28171 7590 07/11/2008</div> <div>ENZO BIOCHEM, INC.</div> <div>527 MADISON AVENUE (9TH FLOOR)</div> <div>NEW YORK, NY 10022</div>				
			<div>EXAMINER</div> <div>RILEY, JEZIA</div>	
			<div>ART UNIT</div> <div>1637</div>	<div>PAPER NUMBER</div>
			<div>MAIL DATE</div> <div>07/11/2008</div>	<div>DELIVERY MODE</div> <div>PAPER</div>

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.



Office Action Summary

Application No.

10/763,088

Applicant(s)

STAVRIANOPOULOS ET AL.

Examiner

Jezia Riley

Art Unit

1637

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --
Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

- 1) ☒ Responsive to communication(s) filed on 09 June 2008.
- 2a) ☐ This action is FINAL. 2b) ☒ This action is non-final.
- 3) ☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

- 4) ☒ Claim(s) 287,290-296 and 299-303 is/are pending in the application.
- 4a) Of the above claim(s) _____ is/are withdrawn from consideration.
- 5) ☒ Claim(s) 296 and 299-303 is/are allowed.
- 6) ☒ Claim(s) 287,290,292 and 293 is/are rejected.
- 7) ☒ Claim(s) 291, 294, 295 is/are objected to.
- 8) ☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

- 9) ☐ The specification is objected to by the Examiner.
- 10) ☐ The drawing(s) filed on _____ is/are: a) ☐ accepted or b) ☐ objected to by the Examiner.
Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).
Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).
- 11) ☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

- 12) ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).
- a) ☐ All b) ☐ Some * c) ☐ None of:
1. ☐ Certified copies of the priority documents have been received.
2. ☐ Certified copies of the priority documents have been received in Application No. _____.
3. ☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

- 1) ☒ Notice of References Cited (PTO-892)
- 2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
- 3) ☐ Information Disclosure Statement(s) (PTO/SB/08)
Paper No(s)/Mail Date _____
- 4) ☐ Interview Summary (PTO-413)
Paper No(s)/Mail Date. _____
- 5) ☐ Notice of Informal Patent Application
- 6) ☐ Other: _____

DETAILED ACTION

Response to Remarks

Applicants' arguments, filed on 6/9/08, have been approved and entered. They have been fully considered. Rejections and/or objections not reiterated from previous office actions are hereby withdrawn. The following rejections and/or objections are either newly applied or reiterated. They constitute the complete set presently being applied to the instant application.

Claim Rejections - 35 USC § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

A person shall be entitled to a patent unless –

(b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

Claims 287, 290, 292, 293, are rejected under 35 U.S.C. 102(b) as being anticipated by Smith et al. (JACS, a983, Vol. 105, pp6638).

Smith et al. discloses a nonmetallic porphyrin comprising an aldehyde moiety as a reactive group attached to the non-pyrrole position. (see page 6639, structure 36). Said porphyrin comprises alkyl group attached to the pyrrole ring, which can comprise further a polar group. It is well known in the art that an aldehyde is capable of forming a carbon-carbon linkage.

Art Unit: 1637

Claims 291, 294, 295 are objected to as being dependent upon a rejected base claim, but would be allowable if rewritten in independent form including all of the limitations of the base claim and any intervening claims.

Claims 296, 299-303 are allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Jezia Riley whose telephone number is 571-272-0786. The examiner can normally be reached on 9:30AM - 5:00PM.

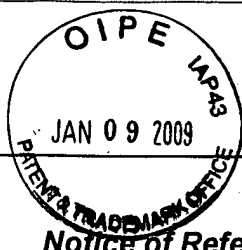
If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Gary Benzion can be reached on 571-272-0782. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see <http://pair-direct.uspto.gov>. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

7/8/2008

/Jezia Riley/

Primary Examiner, Art Unit 1637



Notice of References Cited	Application/Control No. 10/763,088	Applicant(s)/Patent Under Reexamination STAVRIANOPOULOS ET AL.	
	Examiner Jezia Riley	Art Unit 1637	Page 1 of 1

U.S. PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Name	Classification
	A	US-			
	B	US-			
	C	US-			
	D	US-			
	E	US-			
	F	US-			
	G	US-			
	H	US-			
	I	US-			
	J	US-			
	K	US-			
	L	US-			
	M	US-			

FOREIGN PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Country	Name	Classification
	N					
	O					
	P					
	Q					
	R					
	S					
	T					

NON-PATENT DOCUMENTS

*		Include as applicable: Author, Title Date, Publisher, Edition or Volume, Pertinent Pages)
	U	Smith et al. J. Am. Chem. Soc. 1983, 105, pp. 6638-6646.
	V	
	W	
	X	

*A copy of this reference is not being furnished with this Office action. (See MPEP § 707.05(a).)
 Dates in MM-YYYY format are publication dates. Classifications may be US or foreign.

Manipulation of Vinyl Groups in Protoporphyrin IX: Introduction of Deuterium and Carbon-13 Labels for Spectroscopic Studies

Kevin M. Smith,* Eugene M. Fujinari, Kevin C. Langry, Daniel W. Parish, and Hani D. Tabba

Contribution from the Department of Chemistry, University of California, Davis, California 95616. Received March 14, 1983

Abstract: Using commercially available hemin (5) as the starting material, routes for preparation of monovinyl deuterated (27), monovinyl carbon-13 enriched (41, 42), and divinyl carbon-13 enriched (43, 44) derivatives of protoporphyrin IX dimethyl ester (1) are described. The monovinyl carbon-13 enriched porphyrins 41 and 42 were obtained by way of a previously reported Wittig reaction on *Spirographis* and iso-*Spirographis* porphyrin dimethyl esters 28 and 39, respectively. A new efficient partial synthesis of *Spirographis* porphyrin dimethyl ester (28) from deuteroporphyrin IX dimethyl ester (7) is reported, and in this the key formyl group at the 2 position is inserted by way of a Vilsmeier reaction employing a hindered amide.

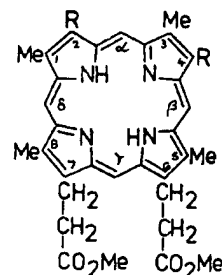
Introduction

Proton NMR spectroscopy has been a particularly important technique for studying the electronic structure of paramagnetic porphyrins and heme proteins.^{1,2} It is to be expected that carbon-13 and deuterium NMR will also contribute significantly to our understanding of the properties of diamagnetic hemes and heme proteins, as well as their paramagnetic counterparts. A significant series of advances in interpretation of the large isotropic NMR shifts³⁻¹⁶ in hemes and heme proteins has been made possible by the availability of regioselectively deuterium-labeled hemes,¹⁷⁻²¹ and in many cases order has been established where a great deal of confusion had existed. More recently, the interpretation of resonance Raman spectra of nickel porphyrins,²² hemes,²³ and heme proteins^{23,24} has been aided by the use of

vinyl-labeled derivatives of protoporphyrin IX dimethyl ester (1).

We report here several methods which have been developed for the regiospecific incorporation of deuterium and carbon-13 labels into the α and β positions of the individual 2- and/or 4-vinyl groups in protoporphyrin IX dimethyl ester. This work introduces an added refinement to the pairwise (2 and 4 position) labeling of vinyl groups with deuterium⁷ or with carbon-13²⁵ which have already been described.

Deuterium Labeling of Vinyl Groups in 1. In our earlier work⁷ we successfully accomplished selective deuteration of the 2- and 4- α -vinyl and 2- and 4- β -vinyl protons in protoporphyrin IX dimethyl ester (1) by carrying out reactions on 2,4-diacetyl-deuteroporphyrin IX dimethyl ester (2). The vinyl-labeled



- 1 R = CH=CH₂
- 2 R = CO₂Me
- 7 R = H
- 40 R = CHO
- 43 R = CH=CH₂
- 44 R = CH=CH₂
- 47 R = CO₂Me
- 48 R = CH(OH)Me

* Indicates carbon-13 enrichment.

compounds enabled us to unequivocally identify the pairs of vinyls in proton NMR and resonance Raman spectra, but it was not possible to uniquely establish which vinyl was which when non-equivalence was unexpectedly identified using these spectroscopic techniques.²⁶ For this purpose we required a protoporphyrin IX

- (1) La Mar, G. N. In "Biological Applications of Magnetic Resonance"; Shulman, R. G., Ed.; Academic Press: New York, 1979; pp 305-343.
- (2) Morrow, J. S.; Gurd, F. R. N. *Crit. Rev. Biochem.* 1975, 3, 221-287.
- (3) Cavaleiro, J. A. S.; Rocha Gonsalves, A. M. d'A.; Kenner, G. W.; Smith, K. M.; Shulman, R. G.; Mayer, A.; Yamane, T. *J. Chem. Soc., Chem. Commun.* 1974, 392-393.
- (4) Mayer, A.; Ogawa, S.; Shulman, R. G.; Yamane, T.; Cavaleiro, J. A. S.; Rocha Gonsalves, A. M. d'A.; Kenner, G. W.; Smith, K. M. *J. Mol. Biol.* 1974, 86, 749-756.
- (5) La Mar, G. N.; Viscio, D. B.; Smith, K. M.; Caughey, W. S.; Smith, M. L. *J. Am. Chem. Soc.* 1978, 100, 8085-8092.
- (6) La Mar, G. N.; Budd, D. L.; Viscio, D. B.; Smith, K. M.; Langry, K. C. *Proc. Natl. Acad. Sci. U. S. A.* 1978, 75, 5755-5759.
- (7) Budd, D. L.; La Mar, G. N.; Langry, K. C.; Smith, K. M.; Nayyar-Mazhir, R. *J. Am. Chem. Soc.* 1979, 101, 6091-6096.
- (8) La Mar, G. N.; Smith, K. M.; Gersonde, K.; Sick, H.; Overkamp, M. *J. Biol. Chem.* 1980, 255, 66-70.
- (9) La Mar, G. N.; Budd, D. L.; Smith, K. M.; Langry, K. C. *J. Am. Chem. Soc.* 1980, 102, 1822-1827.
- (10) La Mar, G. N.; Budd, D. L.; Smith, K. M. *Biochim. Biophys. Acta* 1980, 622, 210-218.
- (11) La Mar, G. N.; de Ropp, J. S.; Smith, K. M.; Langry, K. C. *J. Am. Chem. Soc.* 1980, 102, 4833-4835.
- (12) La Mar, G. N.; de Ropp, J. S.; Smith, K. M.; Langry, K. C. *J. Biol. Chem.* 1980, 255, 6646-6652.
- (13) La Mar, G. N.; de Ropp, J. S.; Smith, K. M.; Langry, K. C. *J. Biol. Chem.* 1981, 256, 237-243.
- (14) La Mar, G. N.; Burns, P. D.; Jackson, J. T.; Smith, K. M.; Langry, K. C.; Strittmatter, P. *J. Biol. Chem.* 1981, 256, 6075-6079.
- (15) La Mar, G. N.; Anderson, R. R.; Budd, D. L.; Smith, K. M.; Langry, K. C.; Gersonde, K.; Sick, H. *Biochemistry* 1981, 20, 4429-4436.
- (16) La Mar, G. N.; Kong, S. B.; Smith, K. M.; Langry, K. C. *Biochem. Biophys. Res. Commun.* 1981, 102, 142-148.
- (17) Cavaleiro, J. A. S.; Rocha Gonsalves, A. M. d'A.; Kenner, G. W.; Smith, K. M. *J. Chem. Soc., Perkin Trans. 1* 1974, 1771-1781.
- (18) Evans, B.; Smith, K. M.; La Mar, G. N.; Viscio, D. B. *J. Am. Chem. Soc.* 1977, 99, 7070-7072.
- (19) Smith, K. M.; Eivazi, F.; Langry, K. C.; Almeida, J. A. P. B.; Kenner, G. W. *Bioorg. Chem.* 1979, 8, 485-495.
- (20) Smith, K. M.; Langry, K. C.; de Ropp, J. S. *J. Chem. Soc., Chem. Commun.* 1979, 1001-1003.
- (21) Smith, K. M.; Langry, K. C. *Int. J. Biochem.* 1980, 12, 689-694.
- (22) Choi, S.; Spiro, T. G.; Langry, K. C.; Smith, K. M. *J. Am. Chem. Soc.* 1982, 104, 4337-4344.

- (23) Choi, S.; Spiro, T. G.; Langry, K. C.; Smith, K. M.; Budd, D. L.; La Mar, G. N. *J. Am. Chem. Soc.* 1982, 104, 4345-4351.
- (24) Rousseau, D. L.; Ondrias, M. R.; La Mar, G. N.; Kong, S. B.; Smith, K. M. *J. Biol. Chem.* 1983, 258, 1740-1746.
- (25) Nelson, M. J.; Huestis, W. H. *Biochim. Biophys. Acta* 1980, 623, 467-470.

derivative with only one of the 2,4-divinyl pair of substituents selectively labeled with deuterium, and subsequently with carbon-13 (see later). By analogy with our previous experience in the divinyl series, it seemed that an approach through selective manipulations of the 2- or 4-substituents in monoacetyldeuteroporphyrin IX derivatives held out the best hope for success in the proposed vinyl differentiation.

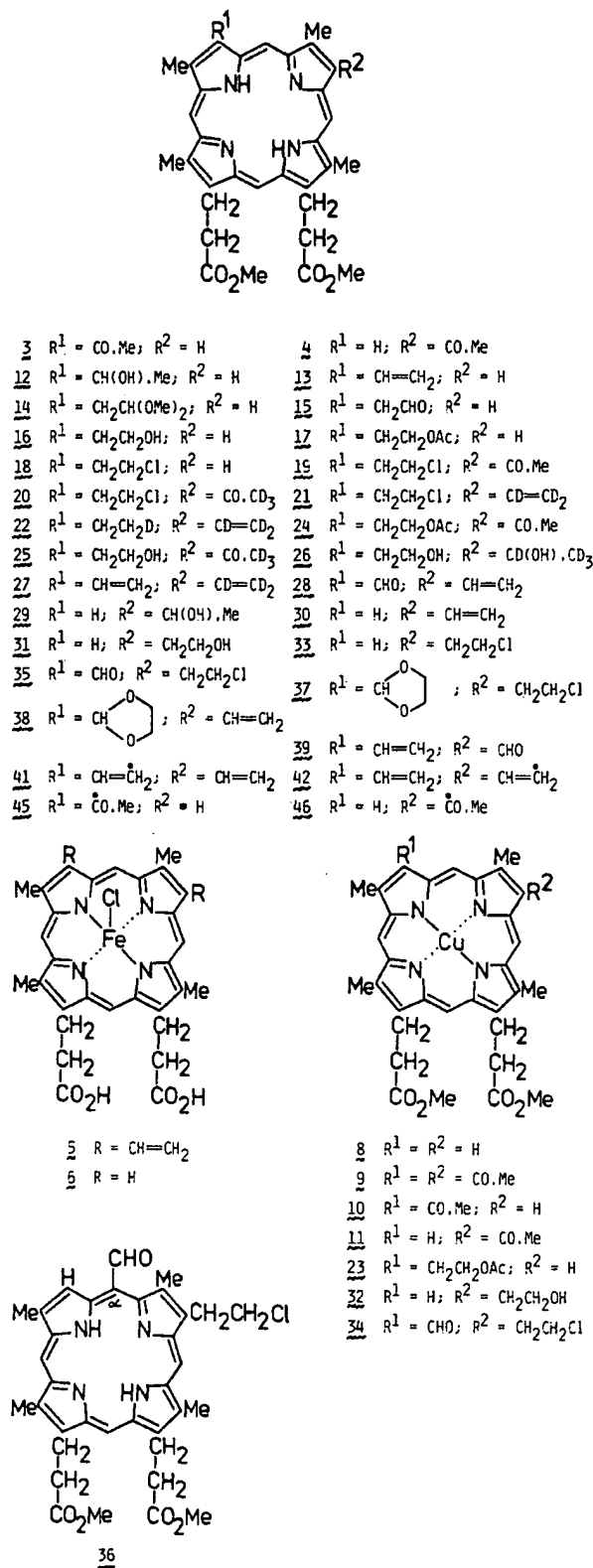
The initial phase of the project required synthesis and separation of large quantities of the monoacetyldeuteroporphyrin IX dimethyl ester isomers 3 and 4. Commercially available hemin (5) was heated in a melt of resorcinol^{27,28} and, following filtration, the crude deuterohemin (6) was subjected to demetalation using the Grinstein procedure²⁹ (FeSO₄/HCl/MeOH). Deuteroporphyrin IX dimethyl ester (7) was obtained in this way in 76% yield from 5.

Although Friedel-Crafts acetylation proceeds well on the iron complex of 7, pilot studies showed that the reaction was cleaner if the copper(II) complex 8 was used instead.²⁸ Acetylation of 8 using acetic anhydride and stannic chloride in dichloromethane gave a mixture containing the copper(II) complexes 9 and 10/11, respectively, of 2,4-diacetyldeuteroporphyrin IX dimethyl ester (2) and the monoacetyl isomers 3/4. These copper(II) complexes were readily separated by column chromatography (unlike the corresponding iron(III) complexes which require prior demetalation), and the copper was removed subsequently by stirring the metalloporphyrins in 10% sulfuric acid in trifluoroacetic acid. After further chromatography, the products (diacetyl 2 and monoacetyls 3/4) were obtained in 67 and 24% yield, respectively, from 8. A minor modification of this procedure, in which the copper(II) deuteroporphyrin IX dimethyl ester (8) was treated at 0 °C for only 20 s gave a separable mixture in which copper(II) monoacetyl isomers 10 and 11 predominated (60% yield, from 8).

The method of separation for the monoacetyl isomers which was initially employed was thick-layer chromatography on silica gel G.³⁰ However, this approach was limited by the small amounts of porphyrin (40 mg) which could be applied to each 20 cm × 40 cm × 1 mm (thick) plate. Since gram quantities of the isomerically pure isomers 3 and 4 were expected to be required for development of the route to mono-vinyl-labeled protoporphyrin IX derivatives, medium-pressure (100–150 psi) liquid chromatography (MPLC) was used. Quantities of between 1 and 2 g of the mixed monoacetyl isomers 10 and 11 could be applied to the Whatman LPS-1 column, and about 80% of the eluted porphyrin was collected as isomerically pure product. The copper(II) 2-acetyldeuteroporphyrin IX dimethyl ester (10) was the more mobile isomer and was generally found in greater abundance than the 4-acetyl isomer 11. Elution of the isomers from the MPLC system usually required about 20 h, and the copper(II) complexes of the porphyrins were used because these seemed to suffer less degradation in that time than the corresponding metal-free porphyrins. Demetalation, as previously, gave the individual pure monoacetyl isomers 3 and 4 from their copper(II) complexes 10 and 11.

Initial plans to accomplish regioselective vinyl labeling revolved around protection of the acetyl function on an iron or copper monoacetyldeuteroporphyrin IX dimethyl ester as the corresponding dithioketal,³² followed by Friedel-Crafts acetylation at

Chart I



(26) NMR⁹ and resonance Raman²¹ data indicate that one vinyl group in myoglobin is significantly more mobile than the other, and NMR studies⁹ have revealed a pH dependence of the oscillatory mobility of vinyl groups in proteins, suggesting that the vinyl hydrogen assignments could be very important for NMR and resonance Raman studies of hemoglobin cooperativity.

(27) Fuhrhop, J.-H.; Smith, K. M. In "Porphyrins and Metalloporphyrins"; Smith, K. M., Ed.; Elsevier: Amsterdam, 1975; p 822.

(28) Brockmann, H., Jr.; Bliesener, K.-M.; Inhoffen, H. H. *Justus Liebig's Ann. Chem.* 1968, 718, 148–161.

(29) Reference 27, pp 802–803.

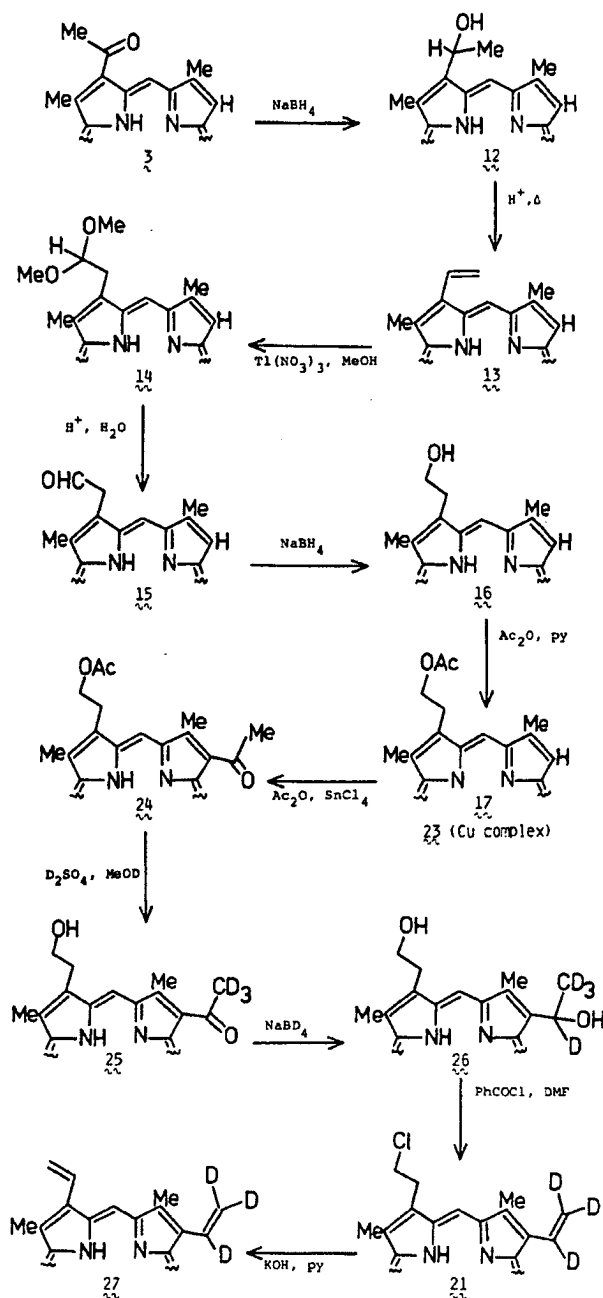
(30) Fractional crystallization³¹ of the mixture was erratic in our hands.

(31) Miller, M. J.; Rapoport, H. *J. Am. Chem. Soc.* 1977, 99, 3479–3485.

(32) Deprotection would probably have occurred during the Friedel-Crafts acylation and workup if the simple ethylene glycol ketal had been employed; we therefore chose a more robust protecting group.

the free position, reduction, and dehydration to give vinyl at this position, and then deprotection and labeling of the regenerated acetyl, as previously described,⁷ to give either α - or β -labeled vinyl. This approach was unexpectedly thwarted by deacetylation of the copper(II) porphyrin under the conditions employed to form the dithioketal;³³ though the dithioketal of the metal-free porphyrin

Scheme I



could be formed, insertion of copper and subsequent Friedel-Crafts acetylation also accomplished deacetylation of the protected acetyl group.

It was therefore decided to protect the two-carbon side chain on the monosubstituted deuterioporphyrin IX in the form of the corresponding 2-chloroethyl; this would not involve a great increase in the number of synthetic steps, and vinyls can be efficiently regenerated from 2-chloroethyls by treatment with base.^{17,34} Vinylporphyrins themselves cannot be subjected to Vilsmeier or Friedel-Crafts reactions owing to unwanted side reactions.³⁵

As shown in Scheme I, 2-acetyldeuterioporphyrin IX dimethyl ester (3) was reduced with sodium borohydride to give the 2-

(1-hydroxyethyl) derivative 12; acid-catalyzed dehydration in *o*-dichlorobenzene³⁶ gave 2-vinyldeuterioporphyrin IX dimethyl ester (isopemptoporphyrin dimethyl ester, 13) in 83% overall yield from 3. Vinyl protection was accomplished using the thallium trinitrate route;³⁷ with 2.2 equiv of thallium(II) trinitrate in presence of methanol, compound 13 gave the corresponding dimethyl acetal 14 after removal of chelated thallium(III). The acetal was hydrolyzed, and the resulting aldehyde, 15, was reduced with sodium borohydride to give 2-(2-(1-hydroxyethyl)deuterio)porphyrin IX dimethyl ester (16) in 87% yield from 13. At this point the 2-(2-(1-hydroxyethyl) group was protected as the corresponding acetate 17; however, prior to this, in an unsuccessful alternate route, the 2-(2-(1-hydroxyethyl)porphyrin 16 was converted into the 2-(2-(1-chloroethyl) derivative 18, subjected to Friedel-Crafts acetylation (acetic anhydride and stannic chloride) to give 19, and then deuterated ($D_2SO_4/MeOD$) to give 20. Sodium borodeuteride reduction, followed by acid-catalyzed dehydration, gave an inseparable mixture of products which was shown by 1H NMR analysis to consist of the required vinylporphyrin 21 and the analogous 2-(2-(1-deuteroethyl)-4-vinylporphyrin 22; the latter compound was obviously produced from 20 by deuterio displacement of the 2-chloro group, and thereby demonstrated that chloroethyl protection of a vinyl group was unsatisfactory in this particular sequence. It was for this reason that we reverted to the 2-(2-(1-acetoxyethyl) protection existing in 17.

Treatment of porphyrin 17 with copper(II) acetate in methanol/dichloromethane gave the copper complex 23 which was subjected to Friedel-Crafts acetylation to give, after removal of the copper (H_2SO_4 /trifluoroacetic acid), a 64% yield of 2-(2-(1-acetoxyethyl)-4-acetyldeuterioporphyrin IX dimethyl ester (24). This was dissolved in $D_2SO_4/MeOD$ and underwent acetyl deuteriation with concomitant methanolysis of the 2-acetate function to give the 2-(2-(1-(1-deutero)hydroxyethyl)-4-acetylporphyrin 25.³⁸ Sodium borodeuteride reduction gave the 2-(2-(1-(1-deutero)hydroxyethyl)-4-(1-(1-hydroxyethyl)porphyrin 26, which, upon treatment with benzoyl chloride and dimethylformamide³⁹ gave a 77% yield of the 2-(2-(1-chloroethyl)-4-vinylporphyrin 21. With sodium hydroxide in pyridine,³⁴ an 83% yield of the required porphyrin, 27, was obtained.

Repetition of the above sequence of reactions, but using the 4-acetyl isomer, 4, would afford the isomeric 2-vinyl-labeled protoporphyrin IX derivative, but for the purposes of the proposed studies, compound 27 alone would be sufficient to definitively differentiate between vinyls 2 and 4 in heme proteins reconstituted with the heme derived from it. Instead, the 4-acetyl material, 4, was used to develop a new partial synthesis of chlorocruoroporphyrin which could be used for carbon-13 enrichment of the 2- β -vinyl carbon in protoporphyrin IX.

Carbon-13 Labeling of Vinyl Groups in 1. Partial Synthesis of Chlorocruoroporphyrin (*Spirographis* porphyrin). Several total⁴⁰ and partial⁴¹ syntheses of chlorocruoroporphyrin have already been reported. Our development of a new partial synthesis of chlorocruoroporphyrin (*Spirographis* porphyrin) dimethyl ester (28) was prompted by the anticipated need (see later) for a formyl-vinyl porphyrin which could be transformed into a specifically labeled 2,4-divinylporphyrin by way of a Wittig reaction using a carbon-13 enriched ylide. We were also interested in demonstrating the

(36) Reference 27, p 771.

(37) Kenner, G. W.; McCombie, S. W.; Smith, K. M. *Justus Liebigs Ann. Chem.* 1973, 1329-1338.

(38) TLC, NMR, and spectrophotometry showed that the major product from this reaction was the corresponding dimethyl ketal of the required acetylporphyrin; this necessitated the inclusion of an extra hydrolysis step.

(39) Clezy, P. S.; Fookes, C. J. R.; Sternhell, S. *Aust. J. Chem.* 1978, 31, 639-648.

(40) (a) Jackson, A. H.; Kenner, G. W.; Wass, J. J. *Chem. Soc., Perkin Trans. 1* 1974, 480-490. (b) Bamfield, P.; Grigg, R.; Johnson, A. W.; Kenyon, R. W. *J. Chem. Soc. C* 1968, 1259-1265. (c) Clezy, P. S.; Diakiv, V. *Aust. J. Chem.* 1975, 28, 2703-2725.

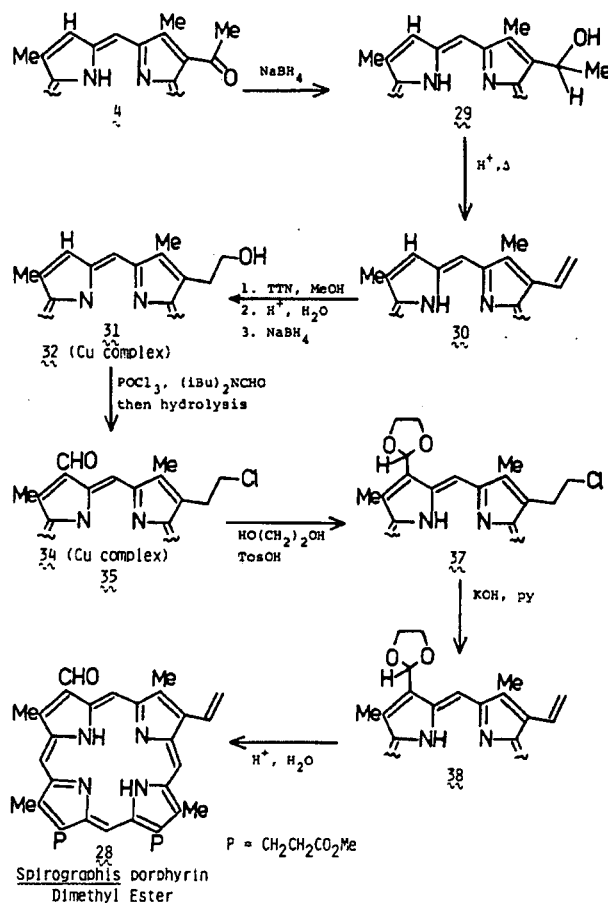
(41) (a) Inhoffen, H. H.; Brockmann, H., Jr.; Bliesener, K.-M. *Justus Liebigs Ann. Chem.* 1969, 730, 173-185. (b) Sono, M.; Asakura, T. *Biochemistry* 1974, 13, 4386-4394. (c) Kenner, G. W.; Quirke, J. M. E.; Smith, K. M. *Tetrahedron* 1976, 32, 2753-2756. (d) Jackson, A. H.; Matlin, S. A.; Rees, A. H.; Towill, R. *J. Chem. Soc., Chem. Commun.* 1978, 645.

(33) Smith, K. M.; Langry, K. C. *J. Chem. Soc. Chem. Commun.* 1981, 283-284.

(34) Clezy, P. S.; Fookes, C. J. R. *Aust. J. Chem.* 1977, 30, 217-220.

(35) E. g., Nichol, A. W. *J. Chem. Soc. C* 1970, 903-910.

Scheme II



synthetic utility of our recently reported⁴² method for formylation at the β positions in porphyrins using a hindered Vilsmeier reagent. Moreover, we were in possession of considerable quantities of 4-acetyldeuterioporphyrin IX dimethyl ester (4) which could be used for these purposes. The successful route is shown in Scheme II.

4-Acetyldeuterioporphyrin IX dimethyl ester (4) was reduced with sodium borohydride, and the resulting 4-(1-hydroxyethyl)porphyrin, 29, was dehydrated using *p*-toluenesulfonic acid in *o*-dichlorobenzene, to give a 75% yield (from 4) of pemtoporphyrin dimethyl ester (30). Application of the thallium(III) trinitrate route (see above) gave an 86% yield of 4-(2-hydroxyethyl)deuterioporphyrin IX dimethyl ester (31) in three steps. The copper(II) complex 32, obtained from 31, was treated with 60 equiv of the preformed phosphoryl chloride/diisobutylformamide complex⁴² in 1,2-dichloroethane. While being heated at 70 °C the reaction mixture was monitored by analytical TLC, and this showed the 2-hydroxyethyl group to be rapidly transformed into 2-chloroethyl, but imine salt formation was much slower. After 7 h, reaction progress seemed to have stopped, so the mixture was hydrolyzed (imine salt-aldehyde) and a thick-layer preparative plate separation afforded three major bands. The most mobile, least abundant band was the copper(II) 4-(2-chloroethyl)porphyrin, which after demetalation gave a 16% yield of 4-(2-chloroethyl)deuterioporphyrin IX dimethyl ester (33). This band was followed by a much slower running green-colored fraction which was presumably a meso-formylated⁴² 4-(2-chloroethyl)porphyrin. Closely trailing this material was the required copper(II) 2-formyl-4-(2-chloroethyl)deuterioporphyrin IX dimethyl ester (34). After demetalation (H_2SO_4 /trifluoroacetic acid), the

2-formyl-4-chloroethylporphyrin, 35, was obtained in 44% yield (or 52% based on recovered 33). NMR spectroscopy of the meso-formylated fraction indicated only one isomer to be present, this probably being the α -meso-formyl-4-(2-chloroethyl)-deuterioporphyrin IX dimethyl ester (36).^{28,42} Treatment of 35 with ethylene glycol and *p*-toluenesulfonic acid gave the acetal 37, which was then dehydrochlorinated with potassium hydroxide in pyridine/methanol to give the vinyl acetal 38. Finally, regeneration of the formyl group by treatment with aqueous acid gave *Spirographis* porphyrin dimethyl ester (28) in 75% overall yield from 35. This material was identical, by melting point and standard spectroscopic techniques, with an authentic sample.

Wittig Reactions on Chlorocruoroporphyrin and Its Isomer. Syntheses of Mono- β -vinyl Carbon-13 Enriched Derivatives of Ptoporphyrin IX Dimethyl Ester. The chlorocruoroporphyrin synthesis described above proved to be too lengthy to apply in large-scale syntheses of 28 and its isomer, 39. These two compounds were therefore synthesized from protoporphyrin IX dimethyl ester (1) using osmium tetroxide/periodate^{41c,43} or by careful ozonolysis,⁴⁴ which afforded a mixture consisting of starting material, 1, the monoformylmonovinylporphyrins 28 and 39, and the corresponding 2,4-diformyldeuterioporphyrin IX dimethyl ester (40). These compounds were separated initially into three fractions (1, 28/39, 40) by column chromatography on alumina, and the monoformyl mixture was then separated using MPLC on Whatman LPS-1 silica; in this final separation, the 2-formyl-4-vinyl isomer 28 was eluted before 39. Identification of the individual isomers was made by melting point, mixture melting point, and finally 360-MHz NMR spectroscopy.

The zinc(II) complexes of compounds 28, 39, and 40 were treated with 99% enriched [^{13}C]methyltriphenylphosphonium iodide in the presence of butyllithium, and afforded 40 to 50% yields of the corresponding vinyl-labeled protoporphyrins IX, 41, 42, and 43, respectively, after removal of the chelating zinc. These samples were identical with authentic protoporphyrin IX dimethyl ester (1), except that in the proton NMR spectrum the vinyl CH_2 resonances experienced a 156–159-Hz ($J_{\text{C-H}}$) coupling in labeled vinyls. In the carbon-13 NMR spectrum an enhanced resonance at ca. 120.9 ppm was observed which, in the proton-coupled mode, was observed as a triplet with $J_{\text{C-H}} \sim 158$ Hz.

Synthesis of Ptoporphyrin IX Dimethyl Ester with Carbon-13 Labels at the α -Vinyl Positions. The most logical approach to this porphyrin, 44, appeared to be via Friedel-Crafts acetylation using 99% ^{13}C enriched acetic acid. On the small scales used, it was most convenient to transform commercially available ^{13}C enriched sodium acetate into acetyl chloride, rather than into acetic anhydride. The acetate was therefore treated with oxalyl chloride and afforded acetyl chloride, ^{13}C enriched, in good yield.⁴⁵

In trial reactions with unlabeled reagents, pure deuterohemin dimethyl ester (6) (obtained by reinsertion of iron into pure deuterioporphyrin IX dimethyl ester, rather than directly from the resorcinol fusion reaction) reacted very much slower with acetyl chloride than with acetic anhydride in the presence of stannic chloride. The major products always appeared to be the iron(III) complexes of the monoacetylated species 3 and 4; the best conditions appeared to involve treatment of deuterohemin dimethyl ester with acetyl chloride and stannic chloride at 0 °C for 12 h, under nitrogen. After a workup in which the iron was removed by the Grinstein procedure,²⁹ column chromatography afforded three bands which were shown to be, in order of increasing polarity, (1) deuterioporphyrin IX dimethyl ester (7) (30% recovery), (2) mixed ^{13}C -labeled monoacetyldeuterioporphyrin IX dimethyl esters (45 and 46), and (3) double labeled 2,4-diacetyldeuterioporphyrin IX dimethyl ester (47). Identities of these compounds were established by melting point comparisons and NMR spectroscopy. The presence of carbon-13 was established by carbon-13 NMR spectroscopy; for example, compound 47 showed one enriched

(43) Sparatore, F.; Mauzerall, D. J. *Org. Chem.* 1960, 25, 1073–1076.

(44) Fuhrhop, J.-H., personal communication.

(45) Kenner, G. W.; Rimmer, J.; Smith, K. M.; Unsworth, J. F. *J. Chem. Soc., Perkin Trans. I* 1977, 332–340.

(42) Smith, K. M.; Langry, K. C. *J. Chem. Soc., Perkin Trans. I* 1983, 439–444.

signal for both labeled carbons around 200 ppm which did not change its multiplicity when run with proton coupling. The 2,4-diacetyldeuteroporphyrin **47** was treated with sodium borohydride to afford the corresponding hematoporphyrin IX, **48**, which was dehydrated, either with *p*-toluenesulfonic acid in *o*-dichlorobenzene³⁶ or with benzoyl chloride and dimethylformamide,³⁹ to give the doubly labeled protoporphyrin IX dimethyl ester derivative **44**. Compound **4**, showed, once again, a single carbon-13 resonance for both side chains at about 62.5 ppm, and in the proton-coupled spectrum this split into two peaks ($J_{C-H} = 140.6$ Hz). In the carbon-13 NMR spectrum of the final product, **44**, one enriched peak appeared at about 130 ppm, and this split into a doublet ($J_{C-H} = 153.4$ Hz) in the proton-coupled spectrum.

Insertion of Iron into the Labeled Porphyrins. Many methods for insertion of iron into porphyrins exist,⁴⁶ but few are suitable for use with sensitive porphyrin and chlorophyll systems. The most widely used method⁴⁷ employs treatment of the porphyrin in a small amount of pyridine with ferrous sulfate in hot acetic acid. In our hands this method can result in decomposition (a base-line spot is almost always observed during TLC analysis) and loss of deuterium labels. Thus, to avoid these losses a new method was developed; this involves addition of a solution of porphyrin in nitrogen-purged chloroform to deoxygenated acetonitrile containing ferrous chloride hydrate.⁴⁸ The method usually calls for an equal weight of ferrous chloride to porphyrin, but there is no doubt that this convenience can be adjusted to use much smaller amounts of the chelating metal. For porphyrins with relatively stable side chains (deuteroporphyrin, protoporphyrin, etc.), the reaction can be performed at 50–60 °C and requires only 15 min. With more sensitive porphyrins, such as deuterium-labeled ones, or hematoporphyrin IX (which suffers dehydration using the usual method⁴⁷), the reaction is conducted at lower temperatures, and takes correspondingly longer. After completion of the reaction (monitored by analytical TLC), the mixture is exposed to oxygen and worked up in the usual way. Carrying out the reaction in acetonitrile usually results in the ferrous chloride being partially in suspension, but acetonitrile can be substituted with methanol as a solvent if this should prove to be troublesome; however, although ferrous sulfate is considerably more soluble in methanol than in acetonitrile, the opposite is true for the porphyrin. Thus, a change to methanol may cause the precipitation of the porphyrin, and since we regard this as a much more serious problem, we prefer to use acetonitrile as the solvent.

Experimental Section

Melting points were measured on a hot-stage apparatus and are uncorrected. Neutral alumina (Merck) was used for column chromatography, and preparative TLC was carried out on 20 × 20 cm glass plates coated with Merck GF 254 silica gel (1 mm thick). Analytical TLC was performed using Merck silica gel 60 F 254 precoated sheets (0.2 mm). Medium-pressure liquid chromatography (MPLC) was carried out using a 2.5 × 100 cm (300 psi rated) glass column (Altex), packed with Whatman silica (LPS-1; 13–24 μ); compounds were eluted at 100–150 psi with a 1/4 in. piston-driven FMI pump, and an ISCO 1840 variable-wavelength detector (set at 405 nm) was used. High-pressure liquid chromatography (HPLC) was performed on a Waters Associates chromatograph, with a Model 6000A solvent delivery system, a U6K injector, a Perkin-Elmer LC 55B variable-wavelength detector (set at 405 nm), and a microporasil (250 × 4.6 mm i.d.) column eluted with tetrahydrofuran or methanol in dichloromethane. Electronic absorption spectra were measured on a Cary 17 spectrophotometer (solutions in dichloromethane), proton NMR spectra were measured at 360 MHz using a Nicolet NT-360 spectrometer, and carbon-13 NMR spectra were measured using a Nicolet NT-200 instrument (solutions in CDCl₃). Mass spectra were measured (direct insertion probe, 70 eV, 50 μ A, source temp. ca. 200 °C) using a Finnegan 3200 mass spectrometer.

Hemin chloride (**5**) was purchased from Man-Win (Washington, D.C.). Elemental analyses were performed at the Berkeley Microanalytical Laboratory, Department of Chemistry, University of California—Berkeley.

2-Acetyl-6,7-bis(2-methoxycarbonyl-ethyl)-1,3,5,8-tetramethylporphyrin (3) and 4-Acetyl-6,7-bis(2-methoxycarbonyl-ethyl)-1,3,5,8-tetramethylporphyrin (4). To 1.92 g of copper(II) deuteroporphyrin IX dimethyl ester in 150 mL of dichloromethane was added 40 mL of acetic anhydride. The mixture was cooled to 0 °C, which caused a substantial amount of the porphyrin-copper complex to precipitate from solution. Freshly distilled stannic chloride (4 mL) was added, and the resulting homogeneous green solution was stirred for 20 s before being poured over 200 g of ice. The mixture was diluted with 200 mL of dichloromethane, and the organic solvent was evaporated under vacuum to give a residue which was chromatographed on neutral alumina (Brockmann Grade III, elution with dichloromethane initially, and then with 0.5% methanol in dichloromethane, to remove diacetylated material). The separated fractions were crystallized from dichloromethane/heptane to give the mixed copper(II) 2- and 4-monoacetyldeuteroporphyrin IX dimethyl esters (**10** and **11**) in a combined yield of 60% (804 mg). The copper(II) complex of 2,4-diacetyldeuteroporphyrin IX dimethyl ester (**2**) was obtained in 27% yield (364 mg).

Separation of the Isomers 10 and 11. Small quantities of the copper(II) monoacetyl isomers (ca. 30–50 mg per plate) could be separated on 20 × 40 cm × 1 mm thick silica gel G preparative TLC plates (elution with 2% tetrahydrofuran in dichloromethane). More convenient large-scale (1–2 g) separations of these compounds **10** and **11** was achieved with medium-pressure (100–150 psi) liquid chromatography (MPLC). With the aid of an infusion pump, ca. 1.5 g of the isomer mixture, dissolved in 30 mL of dichloromethane and contained in a 35-mL plastic syringe, was transferred at a rate of 2.5 mL/min onto a column (2.5 cm × 100 cm) of silica gel (Whatman LPS-1) which had previously been equilibrated with 2% tetrahydrofuran in dichloromethane. Once the solution had been applied to the column, the compounds were eluted with 2% tetrahydrofuran in dichloromethane, a process which usually required 6–10 h before the first fraction began to exit the system. An additional 3–6 h was necessary for the compounds to completely pass through the column; the effluent was collected in 15-mL aliquots using a Gilson microfractionator. Since TLC analysis of the fractions was relatively insensitive, HPLC analysis was used (microporasil column (25 cm × 0.4 cm), eluted with 1% tetrahydrofuran in dichloromethane) to assay the fractions for purity.

Demetalation. To the metalloporphyrins in 30 mL of trifluoroacetic acid was added 3.0 mL of concentrated sulfuric acid, and the mixture was stirred for 1 h at room temperature. The mixtures were then each diluted with 100 mL of dichloromethane and washed with 3 × 100 mL of water; the organic phases were then treated briefly with excess ethereal diazomethane. After evaporation of the solvent, the residues were filtered through a short plug of neutral alumina (Brockmann Grade III, elution with dichloromethane) to give the purified monoacetyl porphyrin free bases. A comparison of the melting point data identified the more chromatographically mobile copper complex as that of the 2-acetyl isomer **10**. In a typical separation, 1.538 g of the mixed copper(II) complexes **10** and **11** gave, after MPLC and demetalation, 0.658 g (47% yield) of the 2-acetylporphyrin **3** and 0.608 g (44% yield) of the 4-acetyldeuteroporphyrin **4**, for a total recovery of 91% of the purified isomers.

Most Mobile Fraction: 2-Acetyldeuteroporphyrin IX Dimethyl Ester (3): mp 238–239 °C (lit.³¹ mp 238.5–240.5 °C); MS, *m/e* (%), 580 (100), 565 (4), 549 (4), 521 (6), 507 (39), 447 (8), 434 (11); ¹H NMR δ (ppm) 3.29 (m, 4 H, CH₂CO), 3.34 (s, 3 H, COMe), 3.56, 3.72, 3.80, 3.93 (each s, 3 H, Me), 3.65, 3.68 (each s, 3 H, OMe), 4.34, 4.48 (each t, 2 H, CH₂CH₂CO), 9.14 (s, 1 H, 4-H), 9.98, 10.03, 10.21, 10.86 (each s, 1 H, meso H).

Least Mobile Fraction: 4-Acetyldeuteroporphyrin IX Dimethyl Ester (4): mp 214–216 °C (lit.³¹ mp 211–213 °C); MS, *m/e* (%), 580 (100), 565 (4), 549 (5), 521 (6), 507 (39), 447 (9), 434 (11); ¹H NMR δ (ppm) 3.28 (q, 4 H, CH₂CO), 3.34 (s, 3 H, COMe), 3.58, 3.72, 3.79, 3.91 (each s, 3 H, Me), 3.66, 3.68 (each s, 3 H, OMe), 4.35, 4.48 (each t, 2 H, CH₂CH₂CO), 9.19 (s, 1 H, 2-H), 10.05, 10.16, 10.83 (each s, 2 H, 1 H, meso H).

6,7-Bis(2-methoxycarbonyl-ethyl)-1,3,5,8-tetramethyl-2-vinylporphyrin, "Isopemptoporphyrin Dimethyl Ester" (13). To 71 mg of 2-acetyldeuteroporphyrin IX dimethyl ester (**3**) dissolved in 40 mL of dichloromethane was added 10 mL of an ice-chilled solution of methanol containing 175 mg of sodium borohydride. The mixture was stirred for 10 min at room temperature, when it was determined by TLC that reduction of the acetyl group was complete. Acetic acid (3 mL) was added to quench excess borohydride, and the solution was diluted with 50 mL of dichloromethane and then washed with 2 × 100 mL of water. The

(46) Buchler, J. W. In ref 27, Chapter 5.

(47) Fuhrhop, J.-H.; Smith, K. M. In ref 27, p 803.

(48) Baldwin and co-workers have developed a similar method using tetrahydrofuran as the solvent: Almog, J.; Baldwin, J. E.; Huff, J. *J. Am. Chem. Soc.* 1975, 97, 227–228. Almog, J.; Baldwin, J. E.; Crossley, M. J.; Debernardis, J. F.; Dyer, R. L.; Huff, J. R.; Peters, M. K. *Tetrahedron* 1981, 37, 3589–3601.

organic fraction was collected and the solvent was evaporated under vacuum. The residue (containing porphyrin 12) was dissolved in 40 mL of *o*-dichlorobenzene containing 180 mg of *p*-toluenesulfonic acid hydrate, and heated at 145 °C for 40 min as nitrogen gas was rapidly passed through the solution. The cooled reaction mixture was diluted with 100 mL of dichloromethane and washed with 3 × 100 mL of water. The vinylporphyrin solution was collected, the solvent evaporated, and the residue dissolved in 50 mL of dichloromethane before being treated with excess ethereal diazomethane. After evaporation of the solvent, the residue was chromatographed on 1 mm thick 20 × 20 cm preparative silica gel TLC plates (elution with 2% methanol in dichloromethane). The major band was collected and the porphyrin was recovered from the silica gel by washing with 3% methanol in dichloromethane. Evaporation of the solvent at reduced pressure, followed by crystallization of the purified porphyrin from dichloromethane/heptane, gave 57 mg (83% yield) of isopentoporphyrin dimethyl ester, mp 219–221 °C (lit.^{40c} mp 219–221 °C). A mixture melting point with authentic material showed no depression: MS, *m/e* (%), 564 (100), 491 (38), 417 (15); ¹H NMR δ (ppm) 3.27 (t, 4 H, CH₂CO), 3.59, 3.63, 3.70, 3.73 (each s, 3 H, Me), 3.64, 3.65 (each s, 3 H, OMe), 4.39 (dt, 4 H, CH₂CH₂CO), 6.17 (dd, 1 H, *cis* β-vinyl CH, *J* = 12 Hz), 6.36 (dd, 1 H, *trans* β-vinyl CH, *J* = 18 Hz), 8.28 (q, 1 H, α-vinyl CH), 9.06 (s, 1 H, 4-H), 9.98, 10.02, 10.07, 10.19 (each s, 1 H, meso H).

6,7-Bis(2-methoxycarbonyl)-1,3,5,8-tetramethyl-4-vinylporphyrin, "Pemptoporphyrin Dimethyl Ester" (30). This compound was similarly prepared from 4-acetyldeuteroporphyrin IX dimethyl ester (4) in 75% yield (73 mg) and had mp 211–213 °C (lit.^{40a} mp 213–214 °C). A mixture melting point with an authentic sample showed no depression: MS, *m/e* (%), 564 (100), 533 (8), 505 (9), 491 (38), 431 (10); ¹H NMR δ (ppm) 3.32 (t, 4 H, CH₂CO), 3.67, 3.73, 3.77 (each s, 12 H, 3 H, 3 H, Me, and OMe), 4.46 (dt, 4 H, CH₂CH₂CO), 6.19 (dd, 1 H, *cis* β-vinyl CH, *J* = 12 Hz), 6.38 (dd, 1 H, *trans* β-vinyl CH, *J* = 18 Hz), 8.32 (q, 1 H, α-vinyl CH), 9.12 (s, 1 H, 2-H), 10.11, 10.14, 10.23 (each s, 2 H, 1 H, 1 H, meso H).

2-(2-Hydroxyethyl)-6,7-bis(2-methoxycarbonyl)-1,3,5,8-tetramethylporphyrin (16). To a solution of 100 mL of dichloromethane and 15 mL of anhydrous methanol containing 510 mg of 2-vinyldeuteroporphyrin IX dimethyl ester (13) was added 884 mg (2.2 mol equiv) of thallium(III) trinitrate trihydrate dissolved in 25 mL of anhydrous methanol. The mixture was stirred for 12 min at 40 °C, and then, after cooling, sulfur dioxide gas was bubbled through the mixture for 1 min. Concentrated hydrochloric acid (1.5 mL) was added and the mixture was stirred for 2 min before the supernatant was decanted and the precipitated thallium(I) salts were washed with additional solvent. The combined organic solutions were washed three times with 100 mL of water and evaporated under vacuum; the residue (compound 14) was dissolved in 100 mL of tetrahydrofuran containing 3 mL of water and 1.5 mL of concentrated hydrochloric acid. This solution was refluxed for 5 min, cooled briefly, diluted with 100 mL of dichloromethane, and then washed twice with water. The solvent was evaporated under vacuum and the residue was dissolved in an arbitrary mixture of tetrahydrofuran/methanol/dichloromethane. The solution of aldehyde 15 was treated at 0 °C with 800 mg of sodium borohydride in 30 mL of ice-cold methanol. It was stirred for 10 min before 3 mL of acetic acid was added to quench excess borohydride. The solvent was evaporated under vacuum and the residue was stirred for 12 h in 150 mL of dry methanol containing 8 mL of concentrated sulfuric acid. The acidic solution was then diluted with 150 mL of dichloromethane and washed with 2 × 150 mL of water. After chromatography on neutral alumina (Brockmann Grade III, elution with chloroform), the red eluates were evaporated and the residue was crystallized from dichloromethane/heptane to give 460 mg (87% yield) of 16: mp 213–215 °C (lit.^{40a} mp 210–212 °C); MS, *m/e* (%), 582 (100), 551 (50), 509 (37), 478 (13), 466 (9), 405 (21); ¹H NMR δ (ppm) 3.28 (t, 4 H, CH₂CO), 3.61, 3.65, 3.68, 3.74 (each s, 3 H, 9 H, 3 H, 3 H, Me, and OMe), 4.40 (m, 8 H, CH₂CH₂CO and CH₂CH₂O), 9.08 (s, 1 H, 4-H), 10.03, 10.10, 10.12 (each s, 1 H, 1 H, 2 H, meso H).

Anal. Calcd for C₃₄H₃₈N₄O₅: C, 70.08; H, 6.57; N, 9.62. Found: C, 70.03; H, 6.58; N, 9.58.

4-(2-Hydroxyethyl)-6,7-bis(2-methoxycarbonyl)-1,3,5,8-tetramethylporphyrin, (31). This compound was likewise prepared from 432 mg of 4-vinyldeuteroporphyrin IX dimethyl ester (30) in 86% yield (384 mg): mp 216–218 °C (lit.^{40a} mp 215–217 °C); MS, *m/e* (%), 582 (100), 581 (65), 551 (30), 509 (28), 405 (15); ¹H NMR δ (ppm) 3.30 (t, 4 H, CH₂CO), 3.66, 3.75 (each s, 15 H, 3 H, Me, and OMe), 4.40 (m, 8 H, CH₂CH₂CO and CH₂CH₂O), 9.10 (s, 1 H, 2-H), 10.06, 10.10, 10.13, 10.15 (each s, 1 H, meso H).

Anal. Calcd for C₃₄H₃₈N₄O₅: C, 70.08; H, 6.57; N, 9.62. Found: C, 69.98; H, 6.69; N, 9.59.

2-(2-Acetoxyethyl)-6,7-bis(2-methoxycarbonyl)-1,3,5,8-tetramethylporphyrin (17). To 310 mg of 2-(2-hydroxyethyl)deuteroporphyrin

IX dimethyl ester (16) in 40 mL of pyridine was added 5 mL of acetic anhydride. The reaction mixture was stirred at room temperature for 20 h and then diluted with 100 mL of dichloromethane and washed with 3 × 100 mL of water. The organic fraction was collected and evaporated to dryness; the residue was chromatographed on neutral alumina (Brockmann Grade III, elution with chloroform). The red eluates were evaporated and the residue was crystallized from dichloromethane/heptane to give 323 mg (97% yield) of the porphyrin acetate: mp 213–215 °C (lit.^{40a} mp 213–215 °C); MS, *m/e* (%), 624 (100), 583 (3), 564 (12), 551 (48), 491 (20), 478 (16); ¹H NMR δ (ppm) 2.01 (s, 3 H, OCOMe), 3.29 (t, 4 H, CH₂CO), 3.65, 3.77 (each s, 15 H, 3 H, Me, and OMe), 4.40 (m, 6 H, CH₂CH₂CO and CH₂CH₂OAc), 4.89 (t, 2 H, CH₂OAc), 9.10 (s, 1 H, 4-H), 10.04, 10.10, 10.13, 10.17 (each s, 1 H, meso H).

Anal. Calcd for C₃₆H₄₀N₄O₆: C, 69.21; N, 6.45; H, 8.97. Found: C, 69.18; H, 6.44; N, 8.99.

2-(2-Acetoxyethyl)-4-acetyl-6,7-bis(2-methoxycarbonyl)-1,3,5,8-tetramethylporphyrin (24). Copper was inserted into porphyrin 17 using copper(II) acetate in methanol/dichloromethane⁴⁹ and afforded a quantitative yield of 23. To 319 mg of this copper complex in 50 mL of dichloromethane was added 10 mL of acetic anhydride. The mixture was cooled to 0 °C in an ice bath and then 1.0 mL of anhydrous stannic chloride was added rapidly. The mixture was stirred for 1 min before being poured over ice. Dichloromethane (50 mL) was added and the mixture was washed with 3 × 100 mL of water before the organic phase was collected and evaporated to dryness under vacuum. To the residue, dissolved in 25 mL of trifluoroacetic acid, was added 2.5 mL of concentrated sulfuric acid, and the mixture was stirred for 1 h at room temperature. The mixture was diluted with 100 mL of dichloromethane and washed with 3 × 150 mL of water. The porphyrin fraction was collected and treated with excess ethereal diazomethane. Following evaporation of the solvent, the residue was chromatographed on 1-mm thick 20 × 20 cm preparative TLC plates of silica gel G (elution with 4% methanol in dichloromethane). The porphyrin was extracted from the silica with 5% methanol in dichloromethane, and evaporation gave a residue which was crystallized from dichloromethane/heptane to give 198 mg (64% yield) of the acetylated porphyrin: mp 204–206 °C; UV-vis, λ_{max} 410 nm (ε 193 000), 510 (10 800), 550 (13 150), 577 (8200), 635 (1750); ¹H NMR δ (ppm) 2.10 (s, 3 H, OCOMe), 3.31 (q, CH₂CO), 3.37 (s, 3 H, COMe), 3.62, 3.72, 3.97 (each s, 3 H, 12 H, 3 H, Me, and OMe), 4.37 (t, 2 H, CH₂CH₂O), 4.48 (q, 4 H, CH₂CH₂CO), 4.93 (t, 2 H, CH₂CH₂O), 10.06, 10.28, 10.87 (each s, 2 H, 1 H, 1 H, meso H).

Anal. Calcd for C₃₈H₄₂N₄O₅: C, 68.45; H, 6.35; N, 8.40. Found: C, 68.36; H, 6.35; N, 8.36.

4-Acetyl-2-(2-hydroxyethyl)-6,7-bis(2-methoxycarbonyl)-1,3,5,8-tetramethylporphyrin (25, Undeuterated). To 91 mg of the foregoing porphyrin, 24, in 10 mL of methanol was added 0.3 mL of concentrated sulfuric acid. After stirring at room temperature for 12 h the solution was diluted with 75 mL of dichloromethane and then washed with 3 × 100 mL of water. The organic layer was collected and evaporated under vacuum to give a residue which was shown by spectrophotometry and ¹H NMR analysis to be the dimethyl ketal of the acetylporphyrin 25. Thus, the porphyrin was dissolved in 10 mL of tetrahydrofuran and stirred with 4 mL of water and 1 mL of concentrated hydrochloric acid for 20 min. The mixture was diluted with 50 mL of dichloromethane and washed with 3 × 100 mL of water before being treated with excess ethereal diazomethane and evaporated to dryness. The residue was chromatographed on neutral alumina (Brockmann Grade IV, elution with chloroform), and the red eluates were evaporated to give a residue which was recrystallized from dichloromethane/heptane to give 80 mg (94% yield) of the required 2-(2-hydroxyethyl)porphyrin: mp 276–278 °C (lit.^{40c} mp 277–279 °C); ¹H NMR δ (ppm) 3.24 (t, 6 H, CH₂OH and CH₂CO), 3.34 (s, 3 H, COMe), 3.60, 3.64, 3.92, 4.42 (6 H, 6 H, 3 H, 3 H, Me, and OMe), 4.35 (m, 6 H, CH₂CH₂CO and CH₂CH₂O), 9.92, 10.06, 10.69, 11.19 (each s, 1 H, meso H).

Anal. Calcd for C₃₆H₄₀N₄O₆: C, 69.21; H, 6.45; N, 8.97. Found: C, 69.34; H, 6.48; N, 9.01.

When the reaction was repeated using D₂SO₄/MeOD, the product, 25 was identical with that described above, except that the ¹H NMR resonance at δ 3.34 was absent (see below).

2-(2-Chloroethyl)-6,7-bis(2-methoxycarbonyl)-1,3,5,8-tetramethyl-4-vinylporphyrin (21, Undeuterated). To 68 mg of the foregoing porphyrin in 40 mL of dichloromethane was added 5 mL of ice-cold methanol containing 75 mg of sodium borohydride. The mixture was stirred for 1 h at room temperature, after which time TLC monitoring indicated reduction of the acetyl group to be complete. The mixture was washed with 30 mL of 0.1 M hydrochloric acid, followed by two washings with 100 mL of water. The organic phase was collected and evaporated to dryness under vacuum; then the residue was dissolved in 10 mL of dry

dimethylformamide, to which was added 1 mL of benzoyl chloride. The mixture was heated at 98 °C for 1.5 h under nitrogen, allowed to cool, and then diluted with 75 mL of dichloromethane and washed with 50 mL of 1 M sodium hydroxide, and then 2 × 75 mL of water. The organic phase was evaporated to dryness and the residue was chromatographed on 1-mm thick 20 × 20 cm preparative silica gel G plates (elution with 2% methanol in dichloromethane). The porphyrin was recovered from the silica by washing with 3% methanol in dichloromethane, which was evaporated, and the residue was recrystallized from dichloromethane/heptane to give 51 mg (74% yield) of the vinylporphyrin: mp 250–252 °C (lit.^{40c} mp 250–252 °C); ¹H NMR δ (ppm) 3.33 (t, 4 H, CH₂CO), 3.69, 3.80 (each s, 15 H, 3 H, Me, and OMe), 4.50 (m, 8 H, CH₂CH₂CO and CH₂CH₂Cl), 6.24 (d, 1 H, cis β-vinyl CH, *J* = 12 Hz), 6.42 (d, 1 H, trans β-vinyl CH, *J* = 18 Hz), 8.31 (m, 1 H, α-vinyl CH), 10.09, 10.14, 10.27 (each s, 1 H, 2 H, 1 H, meso H).

Anal. Calcd for C₃₃H₃₃ClN₄O₄: C, 68.94; H, 6.27; N, 8.93. Found: C, 68.57; H, 6.22; N, 8.81.

2-(2-Chloroethyl)-6,7-bis(2-methoxycarbonyl)-1,3,5,8-tetramethyl-4-(α,β,α-trideuteriovinyl)porphyrin (21). To 91 mg of porphyrin 24 in 10 mL of MeOD was added 9 drops of concentrated D₂SO₄. The mixture was stirred overnight at room temperature in a well-stoppered flask. After dilution with 50 mL of dichloromethane, the mixture was washed with 2 × 50 mL of water, and, as in the unlabeled case, TLC and spectrophotometry indicated the presence of considerable quantities of the dimethyl ketal. The organic phase was therefore evaporated to dryness and the residue was dissolved in 50 mL of tetrahydrofuran containing 4 mL of D₂O and 1 mL of 20% DCl in D₂O. The mixture was stirred for 20 min at room temperature before being diluted with 100 mL of dichloromethane and washed with 2 × 100 mL of water. The organic phase was treated briefly with excess ethereal diazomethane, then evaporated to dryness to give a residue which was dissolved in 40 mL of dichloromethane and treated with 5 mL of ice-cold MeOD containing 75 mg of sodium borodeuteride. Reduction of the acetyl was complete after stirring for 1 h at room temperature, so the solution was diluted with 50 mL of dichloromethane which was washed with 50 mL of 0.1 M hydrochloric acid, followed by 2 × 75 mL of water. The organic fraction was collected, the solvent evaporated under reduced pressure, and the residue dissolved in 10 mL of dry dimethylformamide, to which was then added 1 mL of benzoyl chloride. After heating at 98 °C for 1.5 h under nitrogen, the solution was cooled, diluted with 75 mL of dichloromethane, and then washed successively with 75 mL of 2 M sodium hydroxide and 2 × 100 mL of water. The solvent was removed under vacuum and the residue was chromatographed on 1-mm thick 20 × 20 cm preparative silica gel G TLC plates (elution with 2% methanol in dichloromethane); the principal band was washed from the silica with 3% methanol in dichloromethane, then evaporated to give a red residue which was recrystallized from dichloromethane/methanol to give 66 mg (77% yield) of the deuterated porphyrin, mp 248–250 °C (lit.^{40c} mp 250–252 °C, undeuterated). The ¹H NMR spectrum was identical with that for the unlabeled material described above, except that the resonance at δ 8.31 was absent, and those at δ 6.24 and 6.42 were reduced to 30% intensity, indicating 70% deuteration at the β-vinyl methylene, and 100% deuteration at the α-vinyl methine.

6,7-Bis(2-methoxycarbonyl)-1,3,5,8-tetramethyl-2-vinyl-4-(α,β,β-trideuteriovinyl)porphyrin (27). The foregoing porphyrin 21 (62 mg) was dissolved in 25 mL of pyridine which had previously been refluxed under nitrogen for 30 min; this mixture was then heated under nitrogen at 105 °C and first treated with 4.0 mL of water, followed after 5 min by another 4.5 mL of 3% sodium hydroxide solution. The mixture was stirred at 105 °C for 2.5 h before being cooled to room temperature and diluted with 50 mL of dichloromethane and 50 mL of tetrahydrofuran. The organic phase was washed with 2 × 100 mL of 2 M hydrochloric acid and finally with 100 mL of water. After brief treatment with excess ethereal diazomethane, the solution was evaporated to dryness under reduced pressure and the residue was chromatographed in the dark on 1-mm thick 20 × 20 cm preparative silica gel G TLC plates (elution with 2% methanol in dichloromethane). The major band was removed from the silica with 3% methanol in dichloromethane, which was evaporated to give a red residue, crystallized from dichloromethane/heptane to give 49 mg (83% yield) of the 4-vinyl-labeled protoporphyrin IX dimethyl ester, mp 225–227 °C (lit.⁵⁰ mp 228–229 °C, undeuterated). Apart from peak absences in the ¹H NMR spectrum, this material was identical with an authentic sample of protoporphyrin IX dimethyl ester (1): ¹H NMR δ (ppm) 3.27 (t, 4 H, CH₂CO), 3.60, 3.63, 3.71 (each s, 3 H, 3 H, 6 H, Me), 3.65 (s, 6 H, OMe), 4.40 (t, 4 H, CH₂CH₂CO), 6.19 (d, 1.32 H, cis β-vinyl CH, *J* = 12 Hz, 68% D), 6.37 (d, 1.29 H, trans β-vinyl CH,

J = 18 Hz, 71% D), 8.29 (m, 1 H, α-vinyl CH, 100% D at position 4), 10.04, 10.09, 10.17, 10.23 (each s, 1 H, meso H).

4-(2-Chloroethyl)-2-formyl-6,7-bis(2-methoxycarbonyl)-1,3,5,8-tetramethylporphyrin (35). To 2.0 mL (11.27 mM, 1.77 g) of *N,N*-diisobutylformamide was added rapidly, but dropwise, 100 mL (10.98 mM, 1.68 g) of phosphoryl chloride; the mixture was stirred at room temperature for 40 min. To the resulting viscous imine salt were added 115 mg of copper(II) 4-(2-hydroxyethyl)deuterioporphyrin IX dimethyl ester (32) (obtained in quantitative yield from the free base porphyrin 31 using the copper acetate metal insertion procedure⁴⁹) and 50 mL of 1,2-dichloroethane. The mixture was stirred under nitrogen at 70 °C for 7 h, after which time TLC analysis indicated that the reaction was not proceeding any further. The mixture was therefore cooled in an ice bath and a saturated solution of sodium bicarbonate was added cautiously, after which the mixture was allowed to warm to room temperature, with stirring, overnight. The organic phase was separated, diluted with 50 mL of dichloromethane, and then washed with 2 × 100 mL of water. The organic layer was evaporated under reduced pressure and the residue was chromatographed on 1-mm thick 20 × 20 cm preparative silica gel G plates (elution with 2.5% methanol in dichloromethane), to give three major bands. The most mobile fraction appeared bright red; the least mobile fraction, the most abundant, trailed slightly into the middle band and was green in color. The middle band, clearly separated from the upper fraction, was bright green in color. The bands were recovered from the silica by washing with 5% methanol in dichloromethane, and, following evaporation of the solvent, the residues were demetalated by stirring in 15 mL of trifluoroacetic acid containing 1.5 mL of concentrated sulfuric acid. After 1 h at room temperature, the solutions were diluted with 100 mL of dichloromethane and washed with 3 × 100 mL of water; the separated organic phases were treated with excess ethereal diazomethane. The solvents were removed immediately and the three residues were then chromatographed once more on 1-mm thick 20 × 20 cm preparative silica gel G TLC plates (elution with 2.5% methanol in dichloromethane). After the usual workup, the residues were crystallized from dichloromethane/heptane to give, as the most mobile fraction, 17 mg (16% yield) of 4-(2-chloroethyl)deuterioporphyrin IX dimethyl ester (33). From the middle fraction was isolated 23 mg (20% yield) of what is proposed to be α-meso-formyl-4-(2-chloroethyl)deuterioporphyrin IX dimethyl ester (36). Finally, the least mobile fraction yielded 49 mg (44% yield) of 4-(2-chloroethyl)-2-formyldeuterioporphyrin IX dimethyl ester (35).

Least Mobile Fraction: 4-(2-Chloroethyl)-2-formyldeuterioporphyrin IX Dimethyl Ester (35): mp 229–231 °C (lit.^{40b} mp 228–230 °C); ¹H NMR δ (ppm) 3.27 (m, 4 H, CH₂CO), 3.54, 3.63, 3.70, 3.90 (each s, 3 H, Me), 3.67 (s, 6 H, OMe), 4.31 (t, 4 H, CH₂CH₂Cl), 4.44, 4.53 (each t, 4 H, CH₂CH₂CO), 9.88, 10.00, 10.08, 10.95 (each s, 1 H, meso H), 11.42 (s, 1 H, CHO).

Middle Fraction: α-meso-Formyl-4-(2-chloroethyl)deuterioporphyrin IX Dimethyl Ester (36): mp 189–191 °C; λ_{max} 411 nm (ε 145 100), 510 (8800), 558 (8300), 646 (7500); ¹H NMR δ (ppm) 3.23 (m, 4 H, CH₂CO), 3.50, 3.56, 3.59, 3.63 (each s, 3 H, Me), 3.67, 3.69 (each s, 3 H, OMe), 4.25–4.46 (m, 8 H, CH₂CH₂CO and CH₂CH₂Cl), 9.70 (s, 1 H, 2-H), 9.97, 10.00, 10.12 (each s, 1 H, meso H), 12.54 (s, 1 H, CHO).

Anal. Calcd for C₃₃H₃₇ClN₄O₅: C, 66.82; H, 6.09; N, 8.90. Found: C, 66.93; H, 6.09; N, 8.69.

2-Formyl-4-vinyldeuterioporphyrin IX Dimethyl Ester, "Spirographis Porphyrin Dimethyl Ester" (28). The foregoing 2-formylporphyrin, 35 (72.1 mg), in dry benzene (50 mL) under nitrogen was brought to reflux and then treated with ethylene glycol (1.25 mL) and *p*-toluenesulfonic acid (9.0 mg). Progress of the reaction was followed by spectrophotometry, using the change in absorption spectrum from "rhodo" to "etio" type; the reaction was complete after 45 min. The mixture was diluted with dichloromethane (100 mL), washed with water (3 × 250 mL), dried (Na₂SO₄), and evaporated to dryness to give a residue which was dried further under vacuum. The resulting acetal, 37 (¹H NMR δ (ppm) -3.76 (br s, 2 H, NH), 3.28, 3.29 (each t, 2 H, CH₂CO), 3.61, 3.64, 3.66, 3.67, 3.69, 3.77 (each s, 3 H, Me and OMe), 4.31, 4.38, 4.42, 4.53, 4.55, 4.80 (each t, 2 H, CH₂s in acetal ring, propionates and chloroethyl), 7.39 (s, 1 H, acetal CH), 9.95, 10.07, 10.13, 10.43 (each s, 1 H, meso H)), was taken up in pyridine (30 mL) which had previously been refluxed under nitrogen for 30 min, to which were added water (5.4 mL) and 3% aqueous sodium hydroxide solution (6.4 mL). The solution was refluxed under nitrogen for 2.5 h, before addition of 25% acetic acid in water (6.4 mL), evaporation to a minimum volume, and chasing with toluene to remove most of the pyridine. Addition of water caused precipitation of the crude product (38) which was filtered off, washed with water, and then dissolved in 5% sulfuric acid in methanol (20 mL). After standing overnight, the reaction mixture was worked up by dilution with dichloromethane (200 mL), washing with water (3 × 250 mL), drying

(50) Fischer, H.; Orth, H. "Die Chemie des Pyrrols"; Akademische Verlag: Leipzig, 1937; Vol. II, Part 1, p 401.

(Na_2SO_4), and evaporation to dryness. The residue was crystallized from dichloromethane-methanol to give 51.4 mg (75%) of the required porphyrin, mp 278.5–279.5 °C (lit.^{41a} mp 278 °C). This material was shown, by TLC and mixture melting point analysis, to be identical with an authentic sample of *Spirographis* porphyrin dimethyl ester: λ_{max} 421 nm (ϵ 138 000), 516 (11 400), 556 (15 100), 582 (9400), 642 (2500); ^1H NMR spectrum δ (ppm) –4.35 (br s, 2 H, NH), 3.22 (t, 4 H, $\text{CH}_2\text{CH}_2\text{CO}$), 3.48, 3.55, 3.64, 3.67, 3.72, 3.73 (each s, 3 H, Me and OMe), 4.27, 4.34 (each t, 2 H, $\text{CH}_2\text{CH}_2\text{CO}$), 6.26, 6.40 (each d, 1 H, α -vinyl CH), 8.20 (dd, 2 H, β -vinyl CH_2), 9.65, 9.76, 9.80, 10.55 (each s, 1 H, meso H), 11.23 (s, 1 H, CHO).

Anal. Calcd for $\text{C}_{33}\text{H}_{36}\text{N}_4\text{O}_5$: C, 70.3; H, 6.12; N, 9.45. Found: C, 71.06; H, 6.45; N, 9.08.

2,4-Diformyldeuteroporphyrin IX Dimethyl Ester (40), 2-Formyl-4-vinyldeuteroporphyrin IX Dimethyl Ester ("Spirographis Porphyrin Dimethyl Ester" (28)), and 4-Formyl-2-vinyldeuteroporphyrin IX Dimethyl Ester ("Iso-Spirographis Porphyrin Dimethyl Ester" (39)) by Ozonolysis of Protoporphyrin IX Dimethyl Ester⁴⁴ (1). Protoporphyrin IX dimethyl ester (4 g) in 4.6 L of chloroform and 390 mL of methanol was treated with 8 mL of concentrated sulfuric acid. The resulting deep red solution was cooled to –63 °C (chloroform/dry ice bath), the flask was covered with aluminum foil, and 1 equiv of ozone was passed through the stirred solution. The mixture was stirred for an addition 15 min and then degassed with a stream of nitrogen gas at –63 °C for 45 min. To the stirred mixture were then added 7 g of sodium bicarbonate and 14 mL of dimethyl sulfide, and the mixture was stirred, each time for 1 h, at –63, 0, and finally 20 °C. The chloroform was evaporated under vacuum and the resulting slurry was poured into 1 L of water and extracted with 5 \times 100 mL of chloroform. The combined organic phases were washed with 2 \times 10 mL of water, dried (Na_2SO_4), and then evaporated to dryness. The residue was separated by chromatography on neutral alumina (Brockmann Grade IV, elution initially with dichloromethane, and then with 5% methanol in dichloromethane), and the eluates were evaporated to give, from the least mobile band, 2,4-diformyldeuteroporphyrin IX dimethyl ester, 940 mg (23.4%), recrystallized from dichloromethane/hexane, mp 284–286 °C (lit.⁵¹ mp 284–286 °C). The more mobile band was shown by analytical HPLC (microporous column, elution with 1.2% tetrahydrofuran in dichloromethane) to consist of two closely running monoformylmonovinyl isomers, so the residue was subjected to MPLC at 100 psi (see earlier for description of system) on Whatman LPS-1 silica. The elution solvent was dichloromethane/tetrahydrofuran/isopropyl alcohol (98.4:1.5:0.1), and eluted porphyrins were monitored at 405 nm. The pure isomeric fractions were evaporated to dryness and afforded, as the most mobile fraction, 2-formyl-4-vinyldeuteroporphyrin IX dimethyl ester (*Spirographis* porphyrin dimethyl ester, 28) (80 mg, 2%), crystallized from dichloromethane/hexane, with mp 279 °C (lit.^{41a} mp 278 °C). The least mobile fraction gave 4-formyl-2-vinyldeuteroporphyrin IX dimethyl ester (*iso-Spirographis* porphyrin dimethyl ester, 39), (140 mg, 3.5%), crystallized from dichloromethane/hexane, with mp 225–227 °C, (lit.^{41a} mp 225–227 °C). Analytical HPLC (see above) showed the two isomers to be uncontaminated with each other, and the identities of the individual compounds were established by melting point and mixture melting point comparisons, and finally by 360-Mz ^1H NMR spectroscopy.

6,7-Bis(2-methoxycarbonylethyl)-1,3,5,8-tetramethyl-2,4-bis([^{13}C]-vinyl)porphyrin (43). Zinc was inserted in quantitative yield into 2,4-diformyldeuteroporphyrin IX dimethyl ester (40) using the zinc(II) acetate method.⁴⁹ Dry [^{13}C]methyltriphenylphosphonium iodide (mp 182–184 °C, lit.⁵² mp 187–189 °C, unlabeled) (67 mg) (prepared⁵³ from commercially available 99%-enriched $^{13}\text{CH}_3\text{I}$ and triphenylphosphine) in dry tetrahydrofuran (25 mL) and diisopropylamine (0.55 mL) were treated with *n*-butyllithium in *n*-hexane (0.14 mL, 2.3 M). The resulting ylide was stirred under nitrogen gas and zinc(II) 2,4-diformyldeuteroporphyrin IX dimethyl ester (54 mg) was added. After 5 min the solution was evaporated to dryness to give a residue which was taken into dichloromethane (50 mL), filtered through anhydrous sodium sulfate, and then evaporated again to dryness. The residue was dissolved in trifluoroacetic acid (5 mL) and after 5 min was diluted with dichloromethane (50 mL) and washed twice with water (50 mL). The organic phase was evaporated to dryness and the residue was chromatographed on alumina (Brockmann Grade III, elution with 20% toluene in dichloromethane). The red eluates were evaporated to dryness to furnish a residue which was recrystallized from dichloromethane/methanol to

give 26.9 mg (51%) of the doubly labeled protoporphyrin IX dimethyl ester, mp 208–210 °C (lit.⁵⁴ mp 228–229 °C, unlabeled). The proton NMR spectrum was identical with that of authentic protoporphyrin IX dimethyl ester, except in the vinyl region, where the $=\text{CH}_2$ resonances appeared at 6.18 (dd, J = 11.4 and 159.12 Hz) and 6.37 ppm (dd, J = 17.8 and 156.5 Hz). The carbon-13 NMR spectrum showed a single enhanced resonance at 120.91 ppm, which split into a triplet (J = 158.8 Hz) in the proton-coupled mode.

6,7-Di(2-methoxycarbonylethyl)-1,3,5,8-tetramethyl-2-([^{13}C]-vinyl)-4-vinylporphyrin Dimethyl Ester (41). This porphyrin was likewise prepared from the zinc(II) complex of 2-formyl-4-vinyldeuteroporphyrin IX dimethyl ester (28) (93 mg) and [^{13}C]methyltriphenylphosphonium iodide (93 mg). The product 41 (59.7 mg; 52%) was crystallized from dichloromethane/methanol and had mp 203–205 °C (lit.⁵⁴ mp 228–229 °C, unlabeled). The proton NMR spectrum was identical with that of natural material, except that in the vinyl proton region, one of the vinyl sets of resonances was observed as a carbon-coupled multiplet at 6.28 (dd, J = 11.50 and 158.9 Hz) and 6.39 ppm (dd, J = 18.13 and 156.5 Hz). In the proton-decoupled carbon-13 NMR spectrum a singlet enhanced signal was observed at 120.92 ppm, and in the proton coupled mode this was split into a triplet, J = 157.90 Hz.

6,7-Di(2-methoxycarbonylethyl)-1,3,5,8-tetramethyl-2-vinyl-4-([^{13}C]-vinyl)porphyrin Dimethyl Ester (42). In a similar manner, this carbon-13 monolabeled porphyrin was prepared from the zinc(II) complex of 4-formyl-2-vinyldeuteroporphyrin IX dimethyl ester (39) (108 mg) and 99% enriched [^{13}C]methyltriphenylphosphonium iodide (167 mg). The product (39 mg, 39%) was recrystallized from dichloromethane/methanol and had mp 205–207 °C (lit.⁵⁴ mp 228–229 °C). The proton NMR spectrum was identical with that of authentic protoporphyrin IX dimethyl ester, except that one set of vinyl resonances experienced a carbon-13 coupling at 6.28 (dd, J = 11.63 and 159.00 Hz) and 6.36 ppm (dd, J = 17.8 and 156.30 Hz). The carbon-13 NMR spectrum showed a single enhanced resonance at 120.89 ppm which appeared as a triplet (J = 158.45 Hz) in the proton-coupled mode.

2,4-Di([^{13}C]acetyl)deuteroporphyrin IX Dimethyl Ester (47). Anhydrous (99% enriched) sodium [^{13}C] acetate (2.0 g) was dried at 80 °C (0.5 mmHg) and then suspended in 100 mL of dry dichloromethane; after cooling to 0 °C in an ice bath, 3 mL of oxalyl chloride was added. The suspension was stirred at 0 °C for 3 h, during which time the solid appeared to change in texture. Deuterohemin dimethyl ester (100 mg) was then added, followed by dropwise addition of 2 mL of stannic chloride. The mixture was kept in the dark, under nitrogen at 0 °C for 12 h, and then poured into 100 mL of ice cold water which was extracted with 3 \times 100 mL of chloroform. The combined organic phases were dried (Na_2SO_4) and evaporated to dryness to give a residue which was dissolved in 100 mL of dry methanol containing 1.2 g of powdered ferrous sulfate. Hydrogen chloride gas was bubbled through the solution for 25 min, after which time spectrophotometry showed a pure, two-banded, porphyrin dication spectrum. The solution was therefore cooled in an ice bath and poured into 300 mL of ice-cold water. After extraction with 3 \times 100 mL of chloroform, the extracts were washed successively with water, saturated aqueous sodium bicarbonate, and finally water again before being dried (Na_2SO_4) and evaporated to give a residue. This was chromatographed on neutral alumina (Brockmann Grade III, elution with dichloromethane) and gave three major bands. The least polar band afforded deuteroporphyrin IX dimethyl ester (7) (22 mg, 20%), crystallized from dichloromethane/hexane, with mp 223–224 °C (lit.²⁸ mp 224 °C). The middle band was further purified on 1-mm thick 20 \times 20 cm preparative TLC plates of silica gel (elution with 2% tetrahydrofuran in dichloromethane) and afforded two isomeric [^{13}C]monoacetyldeuteroporphyrins 45 and 46; the less polar of the isomers, after recrystallization from dichloromethane/hexane, mp 236.5–237.5 °C (17 mg, 16%), was identified as the carbon-13 labeled 2-acetyldeuteroporphyrin IX dimethyl ester (45) (lit.³¹ mp 238.5–240.5 °C, unlabeled). The more polar isomer, mp 209.5–210.5 °C, recrystallized also from dichloromethane/hexane (21 mg, 20%), was similarly identified as carbon-13 labeled 4-acetyldeuteroporphyrin IX dimethyl ester (46) (lit.³¹ mp 211–213 °C, unlabeled). The least polar fraction from the alumina chromatography, obtained after changing the elution solvent to chloroform, was identified as doubly labeled 2,4-diacetyldeuteroporphyrin IX dimethyl ester (47), mp 239–240 °C (lit.²⁸ mp 244 °C), and was obtained in 21% yield (22 mg), after recrystallization from dichloromethane/hexane. The carbon-13 NMR spectrum at 50 MHz showed one enriched carbon resonance at 200.00 ppm: ^1H NMR δ (ppm) –3.56 (br s, 2 H, NH), 3.25 (t, 4 H, CH_2CO), 3.7–3.8 (m, 6 H, COMe), 3.58, 3.60, 3.85, 3.90 (each s, 3 H, Me), 3.70 (s, 6 H, OMe), 4.40 (t, 4 H, $\text{CH}_2\text{CH}_2\text{CO}$), 9.80, 9.91, 10.62, 10.80 (each s, 1 H, meso H).

(51) Caughey, W. S.; Alben, J. O.; Fujimoto, W. Y.; York, J. L. *J. Org. Chem.* 1966, 31, 2631–2640.

(52) Hoffmann, H.; Grunewald, R.; Horner, L. *Chem. Ber.* 1960, 93, 861–865.

(53) Monson, R. S. "Advanced Organic Synthesis: Methods and Technique"; Academic Press: New York, 1971; p 105.

(54) Reference 27, p 772.

2,4-Di((1-¹³C)-1-hydroxyethyl)deuterioporphyrin IX Dimethyl Ester (48). The foregoing carbon-13 enriched diacetylporphyrin (20 mg) in 50 mL of chloroform was treated with a solution of 20 mg of sodium borohydride in 2 mL of ice-cold methanol. The mixture was stirred at room temperature for 25 min, after which time it was determined by analytical TLC that reaction was complete. Spectrophotometry also showed a hypsochromic shift in the 639-nm band to 619 nm. Excess hydride was decomposed by addition of 150 mL of 0.04 M hydrochloric acid and the solution was then carefully neutralized with aqueous ammonia. The organic layer was separated and evaporated to dryness to give a residue which was recrystallized from dichloromethane/hexane to give 18 mg (90%) of doubly labeled hematoporphyrin IX dimethyl ester (48), mp 219–220 °C (lit.⁵⁰ mp 223 °C). The carbon-13 NMR spectrum at 50 MHz showed a single enriched peak at 62.50 ppm which became a doublet in the proton-coupled mode, with $J_{C-H} = 140.6$ Hz.

2,4-Di((1-¹³C)vinyl)deuterioporphyrin IX Dimethyl Ester (44). The carbon-13 enriched hematoporphyrin (48) (18 mg) was dissolved in 6 mL of dimethylformamide containing 1 mL of benzoyl chloride and heated at 90 °C for 1.5 h. After this time 2 mL of triethylamine was added to the warm solution, followed by 15 mL of water and 7 mL of methanol. The mixture was shaken to precipitate the product, which was collected by filtration through Celite, and then washed with 10 mL of water and redissolved by addition of 20 mL of chloroform and then collection by suction through the Celite. The organic phase was evaporated and the residue was purified by chromatography on 1-mm thick 20 × 20 cm preparative silica gel TLC plates (elution with 2% methanol in dichloromethane). The porphyrin was recovered from the silica by washing with 3% methanol in dichloromethane, and, after crystallization from dichloromethane/hexane, a quantitative yield of doubly labeled protoporphyrin IX dimethyl ester (44) was obtained, with mp 215–217 °C (lit.⁵⁰ mp 228–229 °C, unlabeled). The carbon-13 ¹H NMR spectrum showed a single enhanced peak at 130.32 ppm, and in the proton-coupled mode the J_{C-H} was 153.4 Hz: ¹H NMR δ (ppm) –3.70 (br s, 2 H, NH), 3.30 (m, 4 H, CH₂CO), 3.68, 3.69, 3.76, 3.77, 3.93 (each s, 3 H, 3 H, 3 H, 6 H, Me, and OMe), 4.23, 4.43 (each m, 2 H, CH₂CH₂CO), 6.21, 6.41 each d, 2 H, CH=CH₂, 8.10, 8.52 (each m, 1 H, CH=CH₂), 10.05, 10.15, 10.21, 10.30 (each s, 1 H, meso H).

Typical Iron Insertion Procedure: Synthesis of Hemin Chloride Dimethyl Ester. To a three-necked, 100-mL round-bottom flask, equipped with a reflux condenser (topped with a nitrogen gas inlet), a pressure-equilibrated dropping funnel (topped with a septum cap), and a nitrogen gas escape tube (with a glycerol bubbler), was added 25 mL of acetonitrile. After refluxing the acetonitrile for 30 min with vigorous stirring (bar magnet) and under a stream of nitrogen, 150 mg of ferrous chloride hydrate was added, and this dissolved with the heat. The mixture was cooled to 50 °C and a solution of 110 mg of protoporphyrin IX dimethyl ester (1) in nitrogen-purged chloroform (12 mL) was transferred through the septum cap into the dropping funnel. Though some of the ferrous salt had precipitated, the porphyrin solution was added to the stirred

ferrous chloride solution at a rate of 2 mL per min. After complete addition, the mixture was stirred for a further 10 min under nitrogen before being exposed to air. The resulting brown solution was then diluted with 125 mL of dichloromethane and washed with 125 mL of 0.2 M hydrochloric acid, and then with 100 mL of water. The organic phase was collected, the solvent evaporated, and the residue sampled by analytical silica gel TLC. The chromatogram indicated that no free base 1 was present and that no ester hydrolysis had occurred. The residue was chromatographed (optional) on neutral alumina (Brockmann Grade III, elution with chloroform containing 0.5% methanol). The major fraction was collected, washed with 0.1 M hydrochloric acid, and then evaporated to dryness to give a brown residue which was crystallized from dichloromethane/heptane to give hemin chloride dimethyl ester (116 mg, 92%), identical by TLC and ¹H NMR spectroscopy (as the cyanoferrin-hemin) with an authentic sample.

Typical Ester Hydrolysis: Synthesis of Hemin (5). The foregoing hemin dimethyl ester (50 mg) was dissolved in 15 mL of a solution made by mixing 95 mL of methanol, 5 mL of water, and 1 g of potassium hydroxide. The solution was refluxed for 5 h at 60 °C under an atmosphere of dry nitrogen. The warm solution was then diluted with 20 mL of methylene chloride and washed with 2 × 50 mL of 0.2 M hydrochloric acid. The organic phase was collected, dried (Na₂SO₄), and evaporated to dryness to give a residue which was recrystallized from tetrahydrofuran to give 40 mg (80% yield) of hemin, identical with an authentic sample by TLC, spectrophotometry, and ¹H NMR spectroscopy (in D₂O, as the dicyanohemin).

Acknowledgment. This research was supported by grants from the National Institutes of Health (HL 22252) and the National Science Foundation (CHE-78-25557). We are pleased to acknowledge financial assistance (to H.D.T.) from Yarmouk University, Jordan.

Registry No. 1, 5522-66-7; 2, 10591-31-8; 3, 13003-76-4; 4, 15295-25-7; 5, 16009-13-5; 8, 14376-24-0; 10, 74822-24-5; 11, 74822-25-6; 12, 63148-21-0; 13, 18210-87-2; 14, 87191-13-7; 15, 87191-14-8; 16, 52459-76-4; 17, 52459-74-2; 21, 87206-75-5; 23, 87191-08-0; 23 acetyl derivative, 87206-74-4; 24, 87191-15-9; 24 dimethyl ketal derivative, 87191-19-3; 25, 87191-17-1; 25 undeuterated, 58684-37-0; 25 dimethyl ketal derivative, 87191-16-0; 26, 87191-20-6; 26 undeuterated, 87191-18-2; 27, 87206-76-6; 27 undeuterated, 58684-43-8; 28, 10200-04-1; 28 zinc complex, 61577-42-2; 29, 15295-26-8; 30, 17467-73-1; 31, 52459-75-3; 32, 87191-09-1; 33, 52459-77-5; 33 copper complex, 87191-11-5; 34, 87191-12-6; 35, 52459-79-7; 36, 87206-77-7; 37, 87191-21-7; 38, 87191-22-8; w39, 13187-15-0; 40, 15341-25-0; 40 zinc complex, 87191-10-4; 41, 5522-66-7; 42, 87191-23-9; 43, 87226-18-4; 44, 87191-26-2; 45, 87191-24-0; 46, 87206-79-9; 47, 87206-78-8; 48, 87191-25-1; deuterio-hemin dimethyl ether, 19442-32-1; sodium [^{1-¹³C}]acetate, 23424-28-4; hemin chloride dimethyl ester, 15741-03-4.

Ab Initio Investigation on the Lowest Singlet and Triplet State of Si₂H₂

Hans Lischka*† and Hans-Joachim Köhler‡

Contribution from the Institut für Theoretische Chemie und Strahlenchemie, Universität Wien, A-1090 Wien, Austria, and Organisations- und Rechenzentrum der Karl Marx Universität Leipzig, DDR-7010 Leipzig, German Democratic Republic. Received February 28, 1983

Abstract: Ab initio SCF and electron correlation calculations are reported for the Si₂H₂ system. In the case of the singlet ground state the global minimum is a nonplanar bridged structure (IV) followed by H₂SiSi (II) and trans-bent HSiSiH (I). Both H₂SiSi and trans-bent HSiSiH are predicted to be local minima. Stability differences ($\Delta H^\circ_{298.15}$) obtained from our most extensive calculations are II/IV 11.8 kcal/mol and I/IV 14.3 kcal/mol. In the triplet case H₂SiSi (II) is the global minimum. Trans bent HSiSiH (I) and a planar bridged structure (III) are local minima. The following stability differences were obtained: I/II 1.7 kcal/mol, III/II 19.7 kcal/mol.

1. Introduction

Compounds containing silicon involved in multiple bonds have always attracted the interest of theoretical and experimental

chemists. However, despite the numerous experimental investigations, direct structural evidence thereof is rather scarce. In recent years theoretical methods have been developed to a degree which allows in many cases (especially for closed-shell ground states) the accurate prediction of structures and stability differences. In the case of silaolefins the interrelation between theory

* Universität Wien.

† Karl Marx Universität.